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=> d his

     (FILE 'HOME' ENTERED AT 15:01:52 ON 17 JUL 2001)

     FILE 'CAPLUS' ENTERED AT 15:01:58 ON 17 JUL 2001
L1         15628 S CELL? (S) SCREEN?
L2           428 S L1 (S) ENZYME?
L3             6 S L2 AND (ENZYME? (S) COMPLEX)
L4          5784 S ENZYME COMPLEX?
L5         47542 S RIBOSOME? OR NUCLEOSOME? OR REPLISOME? OR PROTEOSOME? OR L4
L6          1188 S L5 AND RECOMBINANT
L7            79 S L6 AND SCREEN?
L8            41 S L7 AND CELL?
L9           204 S L6 AND (SCREEN? OR ASSAY?)
L10          110 S L9 AND CELL?
L11          204 S L9 AND (RECOMBIN? OR MUTAGEN? OR MUTAT?)
L12          110 S L10 AND (RECOMBIN? OR MUTAGEN? OR MUTAT?)
L13           26 S L12 AND LIBRAR?
L14           25 S L13 AND SCREEN?
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#8

=> file caplus
=> s enzym?(2w)complex? or complex?(2w)enzym?

676955 ENZYM?
963202 COMPLEX?
11970 ENZYM?(2W)COMPLEX?
963202 COMPLEX?
676955 ENZYM?
3586 COMPLEX?(2W)ENZYM?
L1 14636 ENZYM?(2W)COMPLEX? OR COMPLEX?(2W)ENZYM?

=> s l1 and heterologous or express? or exogenous

18146 HETEROLOGOUS
528233 EXPRESS?
57561 EXOGENOUS
L2 577895 L1 AND HETEROLOGOUS OR EXPRESS? OR EXOGENOUS

=> s l1 and (heterologous or express? or exogenous or chimer?)

18146 HETEROLOGOUS
528233 EXPRESS?
57561 EXOGENOUS
18017 CHIMER?
L3 873 L1 AND (HETEROLOGOUS OR EXPRESS? OR EXOGENOUS OR CHIMER?)

=> s l3 and (cell# or dna)

1457782 CELL#
387552 DNA
L4 404 L3 AND (CELL# OR DNA)

=> d ti 1-200
=> s l1 and (heterologous or exogenous or chimer?)

18146 HETEROLOGOUS
57561 EXOGENOUS
18017 CHIMER?
L5 247 L1 AND (HETEROLOGOUS OR EXOGENOUS OR CHIMER?)

=> d ti 1-247
=> d bib abs hitind 8,11,22,26,31,32,50,51,52,71,80,81,95,99,172,174,199,200

L5 ANSWER 11 OF 247 CAPLUS COPYRIGHT 1998 ACS
AN 1997:672053 CAPLUS

DN 127:343216
TI Engineered intermodular and intramodular polyketide synthase fusions
AU McDaniel, Robert; Kao, Camilla M.; Hwang, Sue J.; Khosla, Chaitan
CS KOSAN Biosciences, Inc., Burlingame, CA, 94010, USA
SO Chem. Biol. (1997), 4(9), 667-674
CODEN: CBOLE2; ISSN: 1074-5521
PB Current Biology
DT Journal
LA English
AB Modular polyketide synthases (PKSs) are very large multifunctional ***enzyme*** ***complexes*** that synthesize a no. of medicinally important natural products. The modular arrangement of active sites has made these enzyme systems amenable to combinatorial manipulation for the biosynthesis of novel polyketides. Here, we investigate the involvement of subunit interactions in hybrid and artificially linked PKSs with several series of intermodular and intramodular fusions using the erythromycin (6-deoxyerythronolide B synthase; DEBS) and rapamycin (RAPS) PKSs. Several two-module and three-module derivs. of DEBS were constructed by fusing module 6 to either module 2 or module 3 at varying junctions. Polyketide prodn. by these intramodular fusions indicated that the core set of active sites remained functional in these hybrid modules, although the ketoreductase domain of module 6 was unable to recognize unnatural triketide and tetraketide substrates. Artificial trimodular PKS subunits were also engineered by covalently linking modules 2 and 3 of DEBS, thereby demonstrating the feasibility of constructing single-chain PKSs. Finally, a series of fusions contg. DEBS and RAPS domains in module 2 of an engineered trimodular PKS revealed the structural and functional tolerance for hybrid modules created from distinct PKS gene clusters. The general success of the intermodular and intramodular fusions described here demonstrates significant structural tolerance among PKS modules and subunits and suggests that substrate specificity, rather than protein-protein interactions, is the primary determinant of mol. recognition features of PKSs. Furthermore, the ability to artificially link modules may considerably simplify the ***heterologous*** expression of modular PKSs in higher eukaryotic systems.

L5 ANSWER 31 OF 247 CAPLUS COPYRIGHT 1998 ACS
AN 1996:410627 CAPLUS
DN 125:78500
TI Use of peptides that bind to one another to direct the interaction of proteins in the formation of multifunctional complexes
IN Pack, Peter; Lupas, Andrei
PA Morphosys Gesellschaft Fuer Proteinoptimierung Mbh, Germany

SO PCT Int. Appl., 33 pp.
CODEN: PIXXD2
PI WO 9613583 A2 960509
DS W: JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 95-EP4117 951020
PRAI EP 94-116558 941020
DT Patent
LA English
AB A method for targeted assembly of distinct active peptide or protein domains into a single complex is described. The method involves incorporating peptide domains and domains that bind specifically to them into proteins that are subunits of the larger complex. The use of a no. of proteins, including ubiquitin, interleukin 2, apomyoglobin, hexahistidine, and decorsin, as the interacting domains in the formation of Ig heterodimers is demonstrated.

L5 ANSWER 32 OF 247 CAPLUS COPYRIGHT 1998 ACS
AN 1996:391166 CAPLUS
DN 125:108093
TI ***Heterologous*** complementation of a Rieske iron-sulfur protein-deficient Saccharomyces cerevisiae by the Rip1 gene of Schizosaccharomyces pombe
AU di Rago, Jean-Paul; Bruel, Christophe; Graham, Laurie A.; Slonimski, Piotr; Trumpower, Bernard L.
CS Dep. Biochem., Dartmouth Med. Sch., Hannover, NH, 03755, USA
SO J. Biol. Chem. (1996), 271(26), 15341-15345
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB A cDNA carrying the Rip1 gene, which encodes the Rieske iron-sulfur protein of Schizosaccharomyces pombe, has been cloned by complementing the respiratory deficiency of a Saccharomyces cerevisiae strain in which the endogenous copy of the RIP1 gene has been deleted. The deduced amino acid sequences of the S. pombe and S. cerevisiae iron-sulfur proteins are 50% identical, with the highest region of identity being in the C termini of the proteins, where the 2Fe:2S cluster is bound. When expressed in the S. cerevisiae deletion strain, the S. pombe iron-sulfur protein restores 25-30% of the ubiquinol-cytochrome c reductase activity. The kinetics of cytochrome c redn., the effects of inhibitors which act at defined sites in the cytochrome bc1 complex, and the optical properties of cytochrome b in membranes from the S. cerevisiae deletion strain complemented with S. pombe iron-sulfur protein indicate that the S. pombe protein interacts with cytochrome b to

restore an apparently normal ubiquinol oxidase site, but that interaction between the iron-sulfur protein and cytochrome c1 is partially impaired. This is the first ***heterologous*** replacement of an electron transfer protein in a respiratory ***enzyme*** ***complex*** in S. cerevisiae.

L5 ANSWER 50 OF 247 CAPLUS COPYRIGHT 1998 ACS
AN 1994:695849 CAPLUS
DN 121:295849
TI The modulation of enzyme reaction rates within multi- ***enzyme*** ***complexes*** . 1. Statistical thermodynamics of information transfer through multi- ***enzyme*** ***complexes***
AU Ricard, Jacques; Giudici-Orticoni, Marie-Therese; Gontero, Brigitte
CS Inst. Jacques Monod, Univ. Paris VII, Fr.
SO Eur. J. Biochem. (1994), 226(3), 993-8
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
AB There is now exptl. evidence that assocn. of different enzymes as a multi- ***enzyme*** ***complex*** may result in an alteration of the catalytic properties of the enzymes present in this complex. This effect is not related to the channelling of reaction intermediates between different active sites. It appears as a consequence of an information transfer that occurs within the multi- ***enzyme*** ***complex*** . A theory, based on statistical thermodn., has been developed which provides an understanding, on a phys. basis, for how isologous as well as ***heterologous*** interactions between identical, or different, ***enzymes*** of the ***complex*** may modulate the catalytic properties of an oligomeric ***enzyme*** of that ***complex*** . The theory predicts three possible types of effects: an alteration, through ***heterologous*** interactions, of an already existing co-operativity of the oligomeric ***enzyme*** within the ***complex*** ; a co-operativity, generated by ***heterologous*** interactions in the complex that could not occur if the oligomeric enzyme were isolated from the rest of the complex; a Michaelis-Menten character of the oligomeric ***enzyme*** within the ***complex*** , but with altered values of Vm and Km relative to what would have been obsd. with the naked enzyme. All these effects appear as a consequence of a transfer of information between different enzymes of the same multi-protein complex. The following paper in this journal shows how one can demonstrate and characterize exptl. these effects in a multi- ***enzyme*** ***complex*** contg. ribulose bisphosphate carboxylase-oxygenase.

L5 ANSWER 52 OF 247 CAPLUS COPYRIGHT 1998 ACS
AN 1994:648005 CAPLUS
DN 121:248005
TI Complementation of Escherichia coli uncD mutant strains by a ***chimeric*** F1-.beta. subunit constructed from E. coli and spinach chloroplast F1-.beta.
AU Burkovski, Andreas; Lill, Holger; Engelbrecht, Siegfried
CS Fach. Biol., Chem. Univ. Osnabrueck, Osnabrueck, D-490069, Germany
SO Biochim. Biophys. Acta (1994), 1186(3), 243-6
CODEN: BBACAQ; ISSN: 0006-3002
DT Journal
LA English
AB ATP-synthesizing F0F1-ATPases are ***complex*** ***enzymes*** consisting of at least eight different subunits. These subunits are conserved during evolution to a very variable degree ranging in pairwise comparison between, for example, Escherichia coli and spinach chloroplast form 20% to 66% identical residues. It was surprising to find that some of the less well conserved subunits like .delta. and .epsilon. could replace their E. coli counterparts, whereas the highly conserved .beta. subunit, which carries the active site, in the E. coli enzyme could not be substituted by spinach chloroplast .beta. (Lill et al. (1993) Biochim. Biophys. Acta 1144, 278-284). We constructed a ***chimeric*** F1-.beta. subunit consisting of spinach .beta. in which the 96 N-terminal amino acids were replaced by the resp. residue sequence from E. coli .beta.. Whereas spinach .beta. did not complement E. coli uncD mutant strains, the ***chimeric*** .beta. subunit restored growth under conditions of oxidative phosphorylation.

L5 ANSWER 81 OF 247 CAPLUS COPYRIGHT 1998 ACS
AN 1992:36999 CAPLUS
DN 116:36999
TI Immobilized fusion proteins as biocatalysts: preparation and use
IN Rudolph, Rainer; Kopetzki, Erhard; Fischer, Stephan; Grossmann, Adelbert; Hoell-Neugebauer, Baerbel
PA Boehringer Mannheim G.m.b.H., Germany
SO Ger. Offen., 13 pp.
CODEN: GWXXBX
PI DE 4001508 A1 910725
AI DE 90-4001508 900119
DT Patent
LA German
AB Biocatalysts are prepd. by expressing ***chimeric*** genes for enzymes fused to binding peptides in host cells, isolating and binding the fusion proteins to a carrier having affinity for the

binding peptide, and using the immobilized biocatalyst for prepn. of a desired product from a substrate. A plasmid encoding .alpha.-glucosidase fused to the hexapeptide Arg6 was prepd. and the ***chimeric*** gene expressed in Escherichia coli. The fusion protein was isolated from the cells and immobilized on Fraktogel EMD SO3--650. The resulting biocatalyst was used to prep. glucose from maltose.

L5 ANSWER 174 OF 247 CAPLUS COPYRIGHT 1998 ACS
AN 1981:402622 CAPLUS
DN 95:2622
TI Complementation in vitro between purified mutant fatty acid synthetase complexes of yeast
AU Werkmeister, Knut; Johnston, Robert B.; Schweizer, Eckhart
CS Univ. Erlangen-Nuernberg, Erlangen-Nuernberg, D-8520, Fed. Rep. Ger.
SO Eur. J. Biochem. (1981), 116(2), 303-9
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
AB By dissocn. and subsequent reassocn. of appropriate pairs of mutant fatty acid synthetases, hybrid multienzyme complexes were obtained whose overall fatty acid synthetase activities were restored to a considerable extent. The complementation thus achieved in vitro could be both intragenic and intergenic and was, in all cases studied, in agreement with the known complementation characteristics of fatty acid synthetase (fas) mutants in vivo. Similarly, the method of reversible dissocn. could be used to reactivate a wild-type fatty acid synthetase which had undergone considerable loss of activity due to prolonged storage. It is believed that only those subunits which have retained their native conformation are used in the reassocn. of the complex. Specific component enzyme activities were detd. with a variety of different mutant fatty acid synthetases and with several hybrid enzymes obtained from them by complementation in vivo or in vitro. Apparently, the activities of most fatty acid synthetase functional domains are influenced by homologous and/or ***heterologous*** subunit interactions within the .alpha.6.beta.6 oligomeric complex. Thus, mutational inactivation of any one of the active sites in subunit .beta. simultaneously also alters the other 4 catalytic sites of this subunit. The lack of complementation occasionally obsd. between certain mutants of 2 different fas 1 complementation groups may be explained by this effect. Furthermore, .alpha./.beta. interactions are indicated by the fact that enoyl-reductase-deficient mutant enzymes (.beta. defect) always exhibit dramatically increased .beta.-ketoacyl synthase (.alpha. domain) activities. The

incorporation of FMN in vitro into wild-type fatty acid synthetase apo-enzyme from which the flavin had been removed leads to a fully active ***enzyme*** ***complex*** only when the cofactor is present during the subunit assembly process. However, addn. of FMN to a fully assocd. apo-enzyme restores only 50% of its original overall sp. activity. If FMN was replaced by FAD in either of these expts., reactivation occurred only to an extent of 50-70% of that obsd. with FMN.

=> s poyketide(w)(synthase? or synthetas?)

```
        1 POYKETIDE
    39790 SYNTHASE?
    34830 SYNTHETAS?
L6      0 POYKETIDE(W)(SYNTHASE? OR SYNTHETAS?)
```

=> s polyketide(w)(synthase? or synthetas?)

```
     1184 POLYKETIDE
    39790 SYNTHASE?
    34830 SYNTHETAS?
L7    318 POLYKETIDE(W)(SYNTHASE? OR SYNTHETAS?)
```

=> s l7 and (heterologous or exogenous or express? or chimer?)

```
    18146 HETEROLOGOUS
    57561 EXOGENOUS
   528233 EXPRESS?
    18017 CHIMER?
L8    107 L7 AND (HETEROLOGOUS OR EXOGENOUS OR EXPRESS? OR CHIMER?)
```

=> d ti 1-107
=> d bib abs hitind 2,5,7,8,12,13,16,17,19-21,26-28,31,34,40-48,61,66,71,72,77,83,92,97

L8 ANSWER 2 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1998:424338 CAPLUS
TI Production of polyketides in bacteria and yeast using multiple vector cloning systems
IN Barr, Philip J.; Santi, Daniel V.; Ashley, Gary W.
PA Kosan Biosciences, USA
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
PI WO 9827203 A1 980625
DS W: AU, CA, JP, NZ
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT,

SE
AI WO 97-US23014 971212
PRAI US 96-33193 961218
DT Patent
LA English
AB Hybrid and novel ***polyketide*** ***synthases*** (PKS) and polyketides are produced by use of a multiple vector system. The combinatorial possibilities offered by placing the various catalytic activities of PKS systems on sep. vectors permits the construction of improved libraries of PKS and polyketides. In addn., polyketides can be produced in hosts that ordinarily do not produce polyketides by supplying, along with an ***expression*** system for the desired PKS, an ***expression*** system for holo ACP synthase. Multivector ***expression*** systems are described for the catalytic activities involved in all of the arom., modular, and fungal PKS systems. Yeast ***expression*** vectors were constructed for the minimal "fungal" PKS encoding 6-methylsalicylic acid synthase (6-MSAS) has only one reading frame for ketosynthase, acyltransferase, a dehydratase, ketoreductase, and acyl-carrier protein, but not a chain length-detg. factor, as well as for holo-ACP synthases (a phosphopantothenoyltransferase) encoded by Bacillus subtilis sfp gene or present in Escherichia coli fatty acid synthase, E. coli enterobactin synthetase, or B. brevis gramicidin synthetase. In addn., 6-deoxyerythronolide B is produced by mixed chromosomal/plasmid systems in Streptomyces lividans using chromosomal integration.

L8 ANSWER 12 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1998:115348 CAPLUS
DN 128:150388
TI Recombinant combinatorial genetic library for the production of novel polyketides
IN Khosla, Chaitan; Hopwood, David A.; Ebert-Khosla, Suzanne; Mcdaniel, Robert; Fu, Hong; Kao, Camilla
PA Leland Stanford Junior University, USA
SO U.S., 53 pp. Cont.-in-part of U.S. Ser. No. 238,811.
CODEN: USXXAM
PI US 5712146 A 980127
AI US 95-486645 950607
PRAI US 93-123732 930920
US 93-164301 931208
US 94-238811 940506
DT Patent
LA English
OS MARPAT 128:150388

AB Novel polyketides and novel methods of efficiently producing both new and known polyketides, using recombinant technol., are disclosed. In particular, a novel host-vector system is described which is used to produce ***polyketide*** ***synthases*** which in turn catalyze the prodn. of a variety of polyketides. The modular gene encoding ***polyketide*** ***synthetase*** enzymes for the synthesis of actinorhodin, granaticin, tetracenomycin, and frenolicin B are cloned into plasmid vectors for recombination and/or directed mutagenesis and ***expression*** in Streptomyces coelicolor. These gene clusters include the open reading frames for ketosynthase/acyltransferase, acyl carrier protein, the chain length detg. factor, polyketide cyclase, polyketide aromatase, ketoreductase, and possibly O-methyltransferases and glycosyltransferases. Rationally designed arom. polyketide synthesis, and the manipulation of macrolide ring size are described.

L8 ANSWER 26 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1997:672053 CAPLUS
DN 127:343216
TI Engineered intermodular and intramodular ***polyketide*** ***synthase*** fusions
AU McDaniel, Robert; Kao, Camilla M.; Hwang, Sue J.; Khosla, Chaitan
CS KOSAN Biosciences, Inc., Burlingame, CA, 94010, USA
SO Chem. Biol. (1997), 4(9), 667-674
CODEN: CBOLE2; ISSN: 1074-5521
PB Current Biology
DT Journal
LA English
AB Modular ***polyketide*** ***synthases*** (PKSs) are very large multifunctional enzyme complexes that synthesize a no. of medicinally important natural products. The modular arrangement of active sites has made these enzyme systems amenable to combinatorial manipulation for the biosynthesis of novel polyketides. Here, we investigate the involvement of subunit interactions in hybrid and artificially linked PKSs with several series of intermodular and intramodular fusions using the erythromycin (6-deoxyerythronolide B synthase; DEBS) and rapamycin (RAPS) PKSs. Several two-module and three-module derivs. of DEBS were constructed by fusing module 6 to either module 2 or module 3 at varying junctions. Polyketide prodn. by these intramodular fusions indicated that the core set of active sites remained functional in these hybrid modules, although the ketoreductase domain of module 6 was unable to recognize unnatural triketide and tetraketide substrates. Artificial trimodular PKS subunits were also engineered by covalently linking modules 2 and 3

of DEBS, thereby demonstrating the feasibility of constructing single-chain PKSs. Finally, a series of fusions contg. DEBS and RAPS domains in module 2 of an engineered trimodular PKS revealed the structural and functional tolerance for hybrid modules created from distinct PKS gene clusters. The general success of the intermodular and intramodular fusions described here demonstrates significant structural tolerance among PKS modules and subunits and suggests that substrate specificity, rather than protein-protein interactions, is the primary determinant of mol. recognition features of PKSs. Furthermore, the ability to artificially link modules may considerably simplify the ***heterologous*** ***expression*** of modular PKSs in higher eukaryotic systems.

L8 ANSWER 27 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1997:667414 CAPLUS
DN 127:330395
TI Biosynthesis of 2-nor-6-deoxyerythronolide B by rationally designed domain substitution
AU Liu, Lu; Thamchaipenet, Arinthip; Fu, Hong; Betlach, Mary; Ashley, Gary
CS KOSAN Biosciences Incorporated, Burlingame, CA, 94010, USA
SO J. Am. Chem. Soc. (1997), 119(43), 10553-10554
CODEN: JACSAT; ISSN: 0002-7863
PB American Chemical Society
DT Journal
LA English
OS CJACS-IMAGE; CJACS
AB The rational prodn. of the erythromycin analog 2-norerythromycin D is described. The methylmalonyl acyltransferase domain in module 6 of the 6-deoxyerythronolide B synthase gene was replaced with the malonyl acyltransferase from module 2 of the rapamycin ***polyketide*** ***synthase*** gene. ***Expression*** of this hybrid gene resulted in formation of 2-nor-6-deoxyerythronolide B and 2-nor-8,8a-deoxyoleandolide, polyketide products arising from extension of propionate and acetate starter units as expected. Feeding of 2-nor-6-deoxyerythronolide B to a culture of an eryA mutant of Saccharopolyspora erythraea gave the antibacterial agent 2-norerythromycin D in high yield. These results show that antibacterial agents can be produced through rational manipulation of ***polyketide*** ***synthases*** ***expressed*** in ***heterologous*** hosts.

L8 ANSWER 28 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1997:665992 CAPLUS
DN 127:292131

TI Manufacture of novel polyketides with transgenic hosts with the native ***polyketide*** ***synthase*** gene cluster partly or completely replaced by foreign synthase genes
IN Khosla, Chaitan; Hopwood, David A.; Ebert-Khosla, Suzanne; McDaniel, Robert; Fu, Hong; Kao, Camilla
PA Leland Stanford Junior University, USA; John Innes Institute
SO U.S., 32 pp. Cont.-in-part of U.S. Ser. No. 164,301.
CODEN: USXXAM
PI US 5672491 A 970930
AI US 94-238811 940506
PRAI US 93-123732 930920
US 93-164301 931208
DT Patent
LA English
AB Novel polyketides and novel methods of efficiently producing both new and known polyketides by ***expression*** of ***polyketide*** ***synthase*** genes in a suitable host. The ***polyketide*** ***synthase*** may be a single type I ***polyketide*** ***synthase*** or it may be a novel species generated by recombining genes for activities of synthases from several species. The genes for these activities may ***expressed*** from their own promoters. The preferred ***expression*** host is a Streptomyces coelicolor strain (CH999) that has had the endogenous actinorhodin synthase cluster (act) deleted. In particular, a novel host-vector system is described which is used to produce ***polyketide*** ***synthases*** which in turn catalyze the prodn. of a variety of polyketides. Genes from the act, gra, fren and tcm ***polyketide*** ***synthase*** clusters were used to construct a series of novel synthase genes that were ***expressed*** in S. coelicolor CH999 with the prodn. of an array of novel polyketides..

L8 ANSWER 31 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1997:588540 CAPLUS
DN 127:293009
TI Combinatorial approaches to polyketide biosynthesis
AU Leadlay, Peter F.
CS Department of Biochemistry and Cambridge Centre for Molecular Recognition, University of Cambridge, Cambridge, CB2 1QW, UK
SO Curr. Opin. Chem. Biol. (1997), 1(2), 162-168
CODEN: COCBF4; ISSN: 1367-5931
PB Current Biology
DT Journal; General Review
LA English
AB A review with 33 refs. Polyketides are a large and structurally

diverse family of natural products based on chains of carboxylic acid units. The ***polyketide*** ***synthases*** that make arom. polyketides have already been used to generate small combinatorial libraries, by ***expressing*** individual genes from different biosynthetic pathways together, so that the enzymes they encode can interact to make novel products. Revent work has shown how to choose these individual components to increase the chances of obtaining such hybrid arom. compds. In other ***polyketide*** ***synthases*** which synthesize complex reduced polyketides, the constituent enzymes are actually linked as domains in a giant multienzyme complex along which the growing polyketide chain is passed. A combinatorial approach here, therefore, requires the fusing together of individual enzymic domains from several such synthases in as many productive ways as can be devised, so that the enzyme assembly line produces a library of altered products. A key recent advance has been to demonstrate that such genuinely hybrid enzymes do work as predicted; for example, a broad-specificity enzyme that recruits the chain starter unit for an antiparasitic compd. has been grafted onto a synthase that makes antimicrobial macrolides.

L8 ANSWER 42 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1997:165227 CAPLUS
DN 126:156479
TI Manufacture of novel polyketides by ***expression*** of foreign ***polyketide*** ***synthase*** genes in a polyketide-synthesizing microbial host
IN Khosla, Chaitan; Hopwood, David A.; Ebert-Khosla, Suzanne; Mcdaniel, Robert; Fu, Hong; Kao, Camilla
PA Leland Stanford Junior University, USA; John Innes Centre
SO PCT Int. Appl., 132 pp.
CODEN: PIXXD2
PI WO 9640968 A1 961219
DS W: AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 96-US9320 960605
PRAI US 95-486645 950607
DT Patent
LA English
OS MARPAT 126:156479
AB A method of manufg. novel and known polyketides by

expression of the genes for ***polyketide*** ***synthetases*** (PKSs) in foreign hosts is described. In particular, a novel host-vector system is described which is used to produce ***polyketide*** ***synthases*** which in turn catalyze the prodn. of a variety of polyketides. The genes may be mutated to produced enzymes with altered properties leading to the formation of novel polyketides. The preferred host is a Streptomyces coelicolor CH999 in which the endogenous actinorhodin PKS gene cluster is replaced. The construction of genes for a no. of ***chimeric*** ***polyketide*** ***synthases*** is described. The structures of the novel polyketides synthesized by these enzymes are used to elucidate the mechanisms of polyketide synthesis by these enzymes. Data from these expts. were used to construct ***chimeric*** ***polyketide*** ***synthases*** designed to catalyze the formation of specific products. The construction of ***polyketide*** ***synthase*** for manuf. of the novel SEK43 is described.

L8 ANSWER 48 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1996:678077 CAPLUS
DN 126:154371
TI A functional ***chimeric*** modular ***polyketide*** ***synthase*** generated via domain replacement
AU Bedford, David; Jacobsen, John R.; Luo, Guanglin; Cane, David E.; Khosla, Chaitan
CS Departments of Chemical Engineering, Stanford Univ., Stanford, CA, 94305-5025, USA
SO Chem. Biol. (1996), 3(10), 827-831
CODEN: CBOLE2; ISSN: 1074-5521
PB Current Biology
DT Journal
LA English
AB Modular ***polyketide*** ***synthases*** (PKSs), such as 6-deoxyerythronolide B synthase (DEBS), are large multifunctional enzymes that catalyze the biosynthesis of structurally complex and medially important natural products. Active sites within these assemblies are organized into 'modules,' such that each module catalyzes the stereospecific addn. of a new monomer onto a growing polyketide chain and also sets the redn. level of the .beta.-carbon atom of the resulting intermediate. The core of each module is made up of a 'reductive segment', which includes all, some, or none of a set of ketoreductase (KR), dehydratase, and enoylreductase domains, in addn. to a large interdomain region which lacks overt function but may contribute to structural stability and inter-domain dynamics within modules. The highly conserved organization of reductive

segments within modules suggests that they might be able to function in unnatural contexts to generate novel org. mols. To investigate domain substitution as a method for altering PKS function, a ***chimeric*** enzyme was engineered. Using a bimodular deriv. of DEBS (DEBS1+TE), the reductive segment of module 2, which includes a functional KR, was replaced with its homolog from module 3 of DEBS, which contains a (naturally occurring) nonfunctional KR. A recombinant strain ***expressing*** the ***chimeric*** gene produced the predicted ketolactone with a yield (35%) comparable to that of a control strain in which the KR2 domain was retained but mutationally inactivated. These results demonstrate considerable structural tolerance within an important segment found in virtually every PKS module. The domain boundaries defined here could be exploited for the construction of numerous loss-of-function and possibly gain-of-function mutants within this family of multifunctional enzymes.

L8 ANSWER 71 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1995:643344 CAPLUS
DN 123:49252
TI Recombinant microorganisms ***expressing*** ***polyketide*** ***synthase*** genes for production of polyketides
IN Khosla, Chaitan; Hopwood, David A.; Ebert-Khosla, Suzanne; McDaniel, Robert; Fu, Hong; Kao, Camilla
PA Leland Stanford Junior University, USA; John Innes Centre
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
PI WO 9508548 A1 950330
DS W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 94-US10643 940920
PRAI US 93-123732 930920
US 93-164301 931208
US 94-238811 940506
DT Patent
LA English
AB Novel polyketides and novel methods of efficiently producing both new and known polyketides, using recombinant technol., are disclosed. In particular, a novel host-vector system is described which is used to produce ***polyketide*** ***synthases*** (PKS's) which in turn catalyze the prodn. of a variety of polyketides. An S. coelicolor strain CH999 genetically engineered to remove the act gene cluster was prepd. Plasmid pRM5 was created to allow ***expression*** of PKS genes in CH999. PRM5 contained

the actII-ORF4 activator gene, the divergent actI/actIII promoter pair, and cloning sites to facilitate insertion of PKS genes downstream of the promoter. Plasmids encoding hybrid PKS's were prepd. using act, gra, and tcm genes. Novel polyketides were prepd. using CH999 transformed with these plasmids.

L8 ANSWER 72 OF 107 CAPLUS COPYRIGHT 1998 ACS
AN 1995:632029 CAPLUS
DN 123:162057
TI Rational design of aromatic polyketide natural products by recombinant assembly of enzymic subunits
AU McDaniel, Robert; Ebert-Khosia, Susanne; Hopwood, David A.; Khosla, Chaitan
CS Dep. Chem. Eng., Stanford Univ., Stanford, NR4 7UH, UK
SO Nature (London) (1995), 375(6532), 549-54
CODEN: NATUAS; ISSN: 0028-0836
DT Journal
LA English
AB Recent advances in understanding of bacterial arom. polyketide biosynthesis allow the development of a set of design rules for the rational manipulation of chain synthesis, redn. of keto groups and early cyclization steps by genetic engineering. The concept of rational design is illustrated by the prepn. of Streptomyces strains that produce two new polyketides, SEK 43 (I) and SEK 26 (II) by ***expression*** of combinations of appropriate enzymic subunits from naturally occurring ***polyketide*** ***synthases*** . The potential for generating mol. diversity within this class of mols. by genetic engineering is enormous.

=> s fatty(w)acid(w)(synthase? or synthetase?)

204377 FATTY
2214287 ACID
39790 SYNTHASE?
34830 SYNTHETASE?
L9 2234 FATTY(W)ACID(W)(SYNTHASE? OR SYNTHETASE?)

=> s l9 and (exogenous or heterologous or chimer? or express?)

57561 EXOGENOUS
18146 HETEROLOGOUS
18017 CHIMER?
528233 EXPRESS?
L10 389 L9 AND (EXOGENOUS OR HETEROLOGOUS OR CHIMER? OR EXPRESS?)

=> s l10 not l8

L11 371 L10 NOT L8

=> s l9 and (exogenous or heterologous or chimer?)

57561 EXOGENOUS
18146 HETEROLOGOUS
18017 CHIMER?
L12 81 L9 AND (EXOGENOUS OR HETEROLOGOUS OR CHIMER?)

=> d ti 1-81
=> d bib abs hitind 8,12,36
=> d ti 1-243
=> d bib abs hitind 5,17,34,59,61,63,67,123,135
L13 ANSWER 59 OF 243 CAPLUS COPYRIGHT 1998 ACS
AN 1997:527072 CAPLUS
DN 127:244302
TI Engineering novel proteins by transfer of active sites to natural ***scaffolds***
AU Vita, Claudio
CS Commissariat l'Energie Atomique, Dep. d'Ingenierie d'Etudes Proteines, CE Saclay, Gif-sur-Yvette, 91190, Fr.
SO Curr. Opin. Biotechnol. (1997), 8(4), 429-434
CODEN: CUOBE3; ISSN: 0958-1669
PB Current Biology
DT Journal; General Review
LA English
AB A review with 36 refs. Novel functional proteins have been generated by the transfer of active sites to structurally homologous proteins and to new structural contexts. The most successful examples of these approaches succeeded in providing effective new tools in biochem. and protein chem. and in suggesting new models in drug design.

L13 ANSWER 61 OF 243 CAPLUS COPYRIGHT 1998 ACS
L13 ANSWER 67 OF 243 CAPLUS COPYRIGHT 1998 ACS
AN 1997:374834 CAPLUS
DN 126:340477
TI ***Enzymic*** array constructed by association of ***enzyme*** -dockerin domain fusion proteins on a ***scaffoldin*** peptide backbone
IN Bott, Richard R.; Clarkson, Kathleen A.; Fowler, Timothy; Liu, Chung-Cheng; Ward, Michael; Xia, Hai-ying
PA Genencor International, Inc., USA

SO PCT Int. Appl., 29 pp.
CODEN: PIXXD2
PI WO 9714789 A2 970424
DS W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, NL, PT, SE
AI WO 96-US16485 961016
PRAI US 95-5701 951017
US 95-559968 951117
DT Patent
LA English
AB An ***enzymic*** array is provided, comprising one or more ***enzymes*** noncovalently bound to a peptide backbone, wherein at least one of the ***enzymes*** is heterologous to the peptide backbone and the peptide backbone is capable of having bound thereto a plurality of ***enzymes*** . A preferred peptide backbone is derived from the CipA or CipB ***scaffoldin*** proteins of Clostridium thermocellum or the CbpA protein of C. cellulovorans, and the ***enzyme*** (s) comprises a fusion with the dockerin domains derived from CelD or CelS cellulase proteins of C. thermocellum. DNAs encoding the CelD and CelS dockerin domains were constructed by assembling synthetic DNA fragments and cloning the assembled fragment in a conventional cloning vector. The recombinant gene encoding the lipase of Pseudomonas mendocino was fused to the dockerin domain-encoding DNAs, and recombinant plasmids transformed into Bacillus subtilis BG3755 for the prodn. of lipase-dockerin fusion protein. CipA ***scaffoldin*** is expressed as a glutathione S-transferase- ***scaffoldin*** fusion protein in Escherichia coli. Significant binding of lipase to cellulose was obsd. only when both the ***scaffoldin*** with intact cellulose-binding domain and dockerin domain were present in the incubation. The array is useful, for example, in recovery systems, targeted multienzyme delivery systems, sol. substrate modification, quantification type assays, and other applications in the food industry, feed, textiles, bioconversion, pulp and paper prodn., plant protection and pest control, wood preservatives, topical lotions, and biomass conversions.
=> log y

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